

## REVIEW ARTICLE

# Uremic toxins in chronic kidney disease highlight a fundamental gap in understanding their detrimental effects on cardiac electrophysiology and arrhythmogenesis

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**Abstract**

Chronic kidney disease (CKD) and cardiovascular disease (CVD) have an estimated 700–800 and 523 million cases worldwide, respectively, with CVD being the leading cause of death in CKD patients. The pathophysiological interplay between the heart and kidneys is defined as the cardiorenal syndrome (CRS), in which worsening of kidney function is represented by increased plasma concentrations of uremic toxins (UTs), culminating in dialysis patients. As there is a high incidence of CVD in CKD patients, accompanied by arrhythmias and sudden cardiac death, knowledge on electrophysiological remodeling would be instrumental for understanding the CRS. While the interplay between both organs is clearly of importance in CRS, the involvement of UTs in pro-arrhythmic remodeling is only poorly investigated, especially regarding the mechanistic background. Currently, the clinical approach against potential arrhythmic events is mainly restricted to symptom treatment, stressing the need for fundamental research on UT in relation to electrophysiology. This review addresses the existing knowledge of UTs and cardiac electrophysiology, and the experimental research gap between fundamental research and clinical research of the CRS. Clinically, mainly absorbents like ibuprofen and AST-120 are studied, which show limited safe and efficient usability. Experimental research shows disturbances in cardiac electrical activation and conduction after inducing CKD or exposure to UTs, but are scarcely present or focus solely on already well-investigated UTs. Based on UTs data derived from CKD patient cohort studies, a clinically relevant overview of physiological and pathological UTs concentrations is created. Using this, future experimental research is stimulated to involve electrophysiologically translatable animals, such as rabbits, or in vitro engineered heart tissues.

**KEYWORDS**

cardiac electrophysiology, cardiorenal syndrome, chronic kidney disease, uremic toxins

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## 1 | INTRODUCTION

Global prevalence of chronic kidney disease (CKD) is estimated at 700–800 million cases worldwide.<sup>1–3</sup> Simultaneously, cardiovascular disease (CVD) is estimated to include 523 million cases,<sup>2</sup> with CVD accounting for approximately 50% of all deaths in CKD patients.<sup>4</sup> In advanced staged CKD patients, the incidence of cardiac arrhythmias and sudden cardiac death (SCD) is estimated around 25%.<sup>5–7</sup> Under pathophysiological conditions, this bidirectional interaction between the heart and kidneys is defined as the cardiorenal syndrome (CRS).<sup>8,9</sup> In CRS, the bidirectional dysfunction of heart and kidneys initiates a cascade of neurohormonal adaptations, hemodynamic changes, inflammation, and oxidative stress, resulting in progressive damage to both organs (Figure 1).<sup>4,10,11</sup> The five ascending stages of CKD emphasize a gradual loss of kidney function, eventually deteriorating to end-stage renal disease (ESRD). Characteristic of CKD progression is the elevation of uremic toxin (UTs) concentrations and other damaging proteins, such as fibroblast growth factors.<sup>12–14</sup> Due to the damaged kidneys, uremic solutes cannot be filtered and excreted sufficiently, with potentially pathological consequences that result from their accumulation.<sup>15</sup> Therefore, ESRD patients are mainly depending on dialysis, as transplantable kidneys are scarcely available. However, during dialysis small molecules diffuse rather quickly when compared to the larger molecules or protein-bound uremic toxins (PBUTs), resulting in the accumulation of these toxins in the blood as they fail to be removed.<sup>16,17</sup>

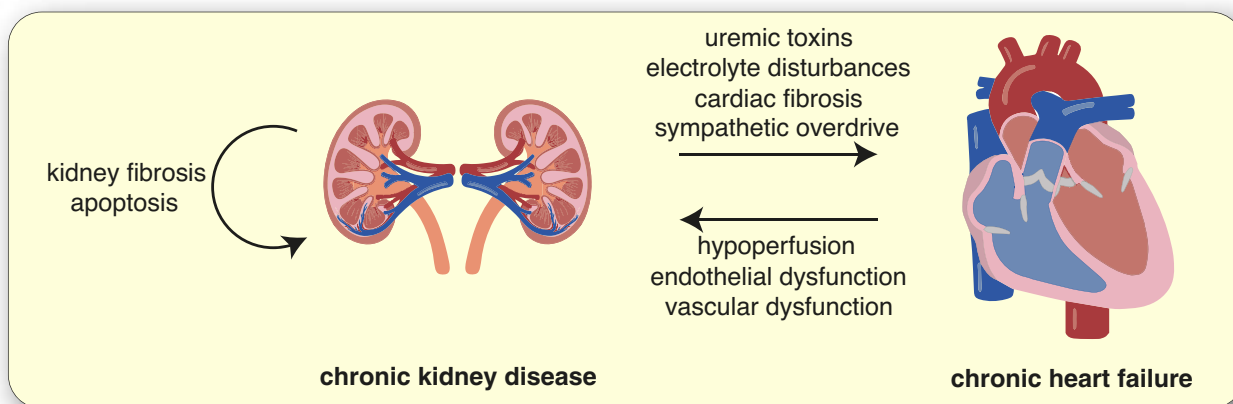
The potential involvement of UTs in cardiac electrophysiological remodeling and its underlying molecular

mechanisms remain poorly understood. Increased incidence of arrhythmias in dialysis patients has also been linked to fluid shifts and fluctuation of potassium concentrations,<sup>5,18</sup> whereas correlations of elevated UT levels with worsened patient outcomes are also shown.<sup>19–21</sup> However, elaborative experimental studies investigating molecular and functional electrophysiological pathology are scarcely performed.

This review discusses electrophysiological research performed on UTs and CKD. First by briefly describing UTs and cardiac electrophysiology, the occurrence of arrhythmias in CKD and the roles of potassium concentrations and the autonomic nervous system. Then, clinically orientated studies will be listed, which mainly focus on improving UT filtering during dialysis in ESRD patients. Subsequently, experimental studies investigating electrophysiological mechanics are discussed, containing both in vivo and in vitro designs. Finally, there is an increasing need for future experimental UT research on cardiac electrophysiology to effectively address the consequences of UTs which ultimately would allow to develop treatment improvements for CKD patients.

## 2 | PROTEIN-BOUND UREMIC TOXINS

In the past decade, UTs were uncovered as important regulators in the CRS, especially PBUTs.<sup>12,15,20</sup> PBUTs are relatively small solutes (<500 Dalton) which have a high affinity to plasma proteins such as albumin. While the majority of research is focused on two PBUTs, being indoxyl sulfate (IS) and p-cresyl sulfate (pCS), many



**FIGURE 1** Progressive co-dysfunction of heart and kidneys defines the cardiorenal syndrome. Chronic kidney dysfunction progressively causes injury to the kidneys, while also leading to accumulation of uremic toxins and electrolyte disturbances, worsening functionality of the heart. Continuous dysfunction of the heart subsequently can cause vascular damage and hypoperfusion in the kidneys, further establishing this vicious cycle of disease.

other PBUTs exist.<sup>22</sup> Most of the PBUTs are liver or intestinal-derived metabolic products that are released into the circulation and subsequently filtered from the blood by the kidneys.<sup>23</sup> Several review manuscripts have been published describing the pathological effects of selected UTs in both heart and kidney, including increased inflammation, fibrosis formation, oxidative stress, and hypertrophy.<sup>12,23,24</sup>

UTs, specifically IS, have been shown to increase both gene expression and protein levels of tumor necrosis factor alpha (TNF- $\alpha$ ) and the interleukins 1 $\beta$  and 6 (IL-1 $\beta$  and IL-6).<sup>25,26</sup> Additionally, elevated gene expression of transforming growth factor beta (TGF- $\beta$ ) and connective tissue growth factor (CTGF) substantiated the increased fibrosis formation in the heart, as well as in the kidneys.<sup>23,27</sup> Markers of myocardial hypertrophy, such as  $\alpha$ -skeletal muscle actin and  $\beta$  myosin heavy chain, are also increased in nephrectomized rats and isolated cardiomyocytes.<sup>27,28</sup> All these processes are activated by signaling nodes that involve the nuclear factor kappa-light-chain-enhancer of activated B cells (NF $\kappa$ B) and mitogen-activated protein kinase (MAPK),<sup>26-28</sup> as a consequence of elevated oxidative stress.<sup>23,29,30</sup> Although the effects evoked through elevation of individual concentrations of UTs are not often investigated in animal studies, as the serum contains an array of UTs and non-standardized concentrations, these pathological effects are directly associated with the detrimental activity of elevated levels of at least IS, pCS, but also indole-3 acetic acid (IAA).<sup>31</sup>

## 2.1 | Uremic concentrations in patients

Based on studies implementing clinical cohorts,<sup>24,32-34</sup> as well as a comprehensive literature analysis by the European Uremic Toxin Work Group,<sup>14,22</sup> concentration ranges can be made of physiological and pathological UT levels. While increasing UT concentrations can already be measured in early CKD stages,<sup>32</sup> serum analysis is mainly performed in ESRD and dialysis patients. Based on these datasets, an overview of several PBUTs and their respective total concentrations was designed (Figure 2). Both physiological (shown in green) and pathological (shown in red) concentrations can highly vary between patients, complicating the establishment of a definitive toxic concentration of most UTs. The UTs with the highest pathological concentrations, including IS, pCS, and hippuric acid (HIP), also claim the majority of recognition in the scientific research field. This also accounts for the non-UT fibroblast growth factor 23 (FGF23). However, it could be advantageous to investigate UTs of which the physiological and pathological concentrations are relatively close, as the border to toxicity is crossed more easily.

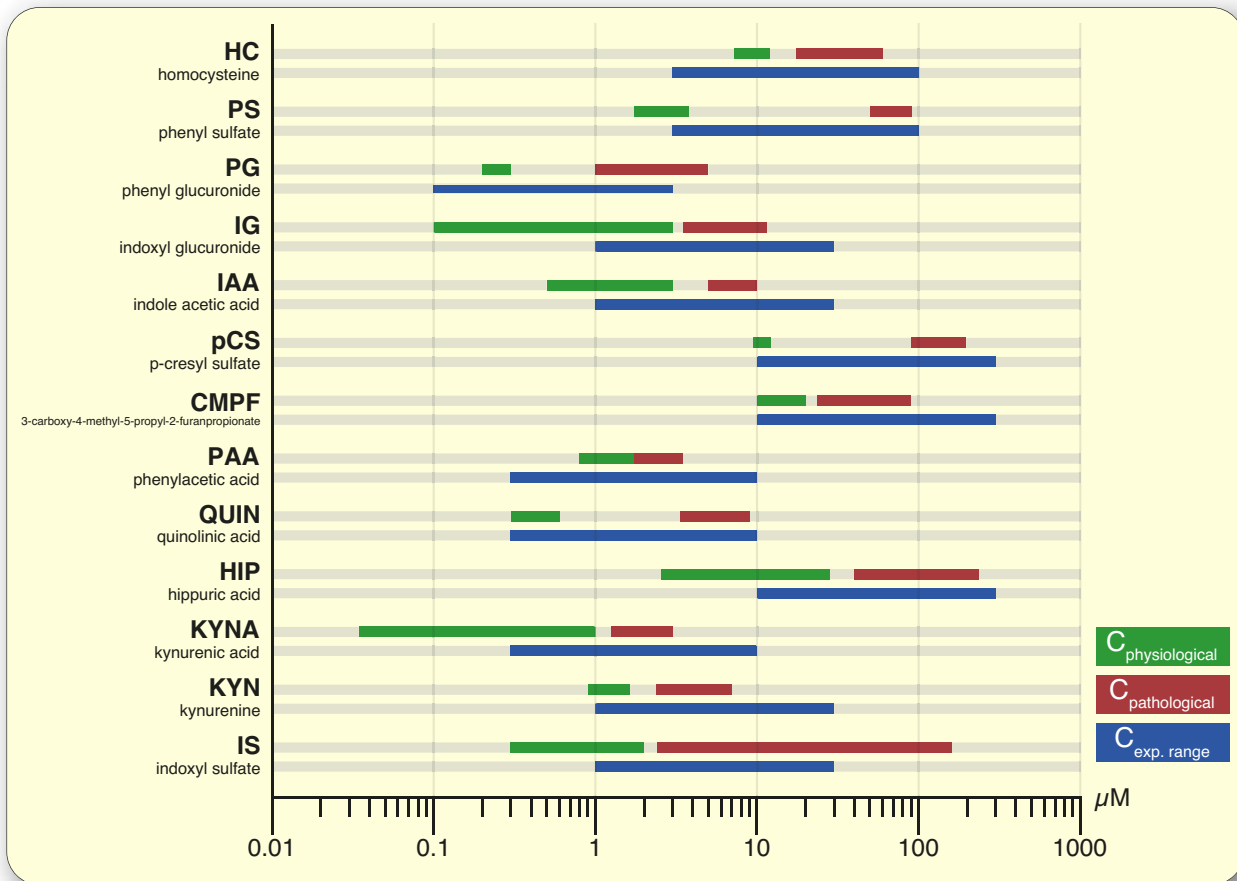
## 3 | CARDIAC ELECTROPHYSIOLOGY AND ARRHYTHMIAS

Alterations in cardiac electrophysiology underlying increased arrhythmic risk can be investigated at different levels. The initiation and morphology of cardiac action potentials (APs), the intracellular calcium homeostasis, or the propagation of the APs from cell to cell can all be disturbed. Establishing the influence of PBUTs on any of the molecular pathways is valuable to understand the pro-arrhythmic risk in CKD patients. In the past decade, the atria have been the most intensively investigated tissue regarding the relation between elevated UTs and arrhythmias, as the prevalence of atrial fibrillation (AF) is at least twice as high in CKD patients compared to non-CKD populations,<sup>21,35</sup> and especially, this applies to the dialysis population.<sup>36-38</sup>

### 3.1 | Action potentials, calcium transients, and impulse propagation

The cardiac action potential is the summation of multiple ion currents, originating from ions passing the cell membrane through specific ion channels, where shortening or prolongation of the action potential duration (APD) is the consequence of alterations in density of these currents (Figure 3).<sup>39</sup> The resting membrane potential of a cardiomyocyte is mainly stabilized by the inward rectifier potassium current ( $I_{K1}$ ) and is the main characteristic influencing excitability of a single cell (phase 4). Influx of Na<sup>+</sup> through sodium channels ( $I_{Na}$ ) rapidly depolarizes the cell membrane, which is the first step of the AP (phase 0). The transient outward potassium current ( $I_{to}$ ) causes a small period of initial repolarization (phase 1), directly followed by the influx of Ca<sup>2+</sup> ( $I_{Ca,L}$ ), which temporarily leaves the membrane potential at a plateau phase because of a balance due to the simultaneous initiation of potassium efflux currents (phase 2). Further conductance of these rapid and slow delayed rectifier potassium currents ( $I_{Kr}$  and  $I_{Ks}$ , respectively) repolarizes the membrane potential (phase 3), which is ultimately accomplished by the  $I_{K1}$  current that brings the cell back to its initial resting membrane potential. The sodium/calcium exchanger (NCX) and the sodium/potassium ATPase (NKA) both support  $I_{K1}$  in maintaining the resting membrane potential until the next depolarization (phase 4).

Simultaneously its role in the plateau phase,  $I_{Ca,L}$  triggers the Ca<sup>2+</sup> release from the sarcoplasmic reticulum (SR) via activation of the ryanodine receptor (RyR), a phenomenon which is described as calcium-induced calcium release (phase A). The subsequent large increase in



**FIGURE 2** Overview of clinically relevant experimental total uremic toxin concentrations. Physiological (in green) and pathological (in red) serum concentrations of uremic toxins in patients vary extremely, but can be used to create an experimental (in blue) concentration range that spans both conditions. Using these concentrations, experiments can be designed to specifically define pathologic concentrations for individual uremic toxins.

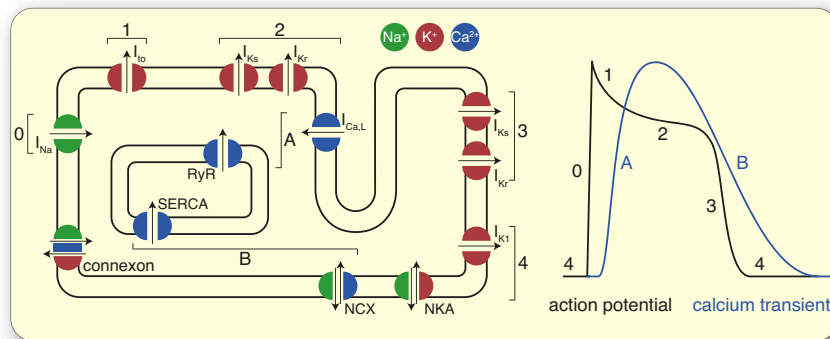
cytosolic  $\text{Ca}^{2+}$ , reflected through the calcium transient (CaT), facilitates the calcium-dependent contraction of the sarcomeres. Lowering cytosolic  $\text{Ca}^{2+}$  during relaxation is mainly achieved by pumping the  $\text{Ca}^{2+}$  back into the SR, via the sarco/endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase (SERCA), or out of the cell via NCX (phase B).<sup>40</sup>

Propagation of APs between cardiomyocytes facilitates the organized contraction of the myocardium. Pathophysiological remodeling resulting in an increased heterogeneity between cardiomyocytes, either in electrical or mechanical coupling, can affect conductivity.<sup>41</sup> The specific subcellular region that regulates electromechanical coupling between cardiomyocytes is named the intercalated disk (ID).<sup>42,43</sup> The ID consists of adherens junctions and desmosomes, which provide structural support between cardiomyocytes and gap junctions, that provide electrical and metabolic coupling of cardiomyocytes. The gap junctions are formed by two connexon hemichannels, each one delivered by the two connecting cells. These

connexons are composed of six connexin (Cx) proteins, with Cx43 being the main isoform found in the ventricular myocardium.<sup>44</sup> The normal spatio-temporal pattern of conduction throughout the heart is initiated in the sinus node in the right atrium and runs via the atrio-ventricular node, through the ventricular septum, towards the free wall of both ventricles. The subsequent electrical activation of different parts of the heart in space and time is represented in an electrocardiogram (ECG), in which the direction and intensity of the electrical activity are shown in respect to electrodes attached to body.

### 3.2 | Arrhythmias

Impaired integrity of the protein complexes at the ID and a reduction in intercellular electrical communication hampers coupling causing a decreased conductivity across the myocardium. Increased fibrosis formation, consisting



**FIGURE 3** Representation of cardiac ion currents, orchestrating the morphology of action potentials and calcium transients. The cardiac action potential starts with an inflow of sodium ions ( $I_{Na}$ ) depolarizing the cardiomyocyte (phase 0). As the sodium channels inactivate, the transient outward potassium current ( $I_{to}$ ) causes an initial repolarization (phase 1). This is followed by the influx of calcium ions ( $I_{Ca,L}$ ), as well as an efflux of potassium ions ( $I_{Kr}$  and  $I_{Ks}$ ), which brings the membrane potential at a plateau (phase 2). Further conductance of  $I_{Kr}$  and  $I_{Ks}$  strongly repolarizes the membrane potential (phase 3). Finally, the cell is brought to a resting membrane potential by the inward rectifier potassium current ( $I_{K1}$ ), with support of the sodium/calcium exchanger (NCX) and the sodium/potassium pump (phase 4). Simultaneously to causing the plateau phase,  $I_{Ca,L}$  triggers the calcium release from the sarcoplasmic reticulum (SR) via activation of the ryanodine receptor (RyR), a phenomenon which is described as calcium-induced calcium release, reflected through the rise of a calcium transient (phase A). Decay of cytosolic calcium is mainly achieved by pumping the calcium back into the SR, via the sarco/endoplasmic reticulum  $Ca^{2+}$  ATPase (SERCA), or out of the cell via NCX (phase B). Propagation of action potentials to neighboring cells is partly facilitated by connexon hemichannels.

of relatively poorly conductible tissue, can electrically insulate cardiomyocytes, which forms physical conduction barriers in addition to slowing intercellular AP propagation.<sup>45</sup> This might lead to a conduction path that no longer follows the traditional propagation route, but rather disperses autonomously and repeatedly through a limited region, which is named a reentry arrhythmia.

Ion channel expression and protein levels impeded intracellular trafficking and insertion into the sarcolemma, and channel kinetics or blockade all impact current densities, thereby influencing the morphology of the AP. An important example of channels that are sensitive to modulation are channels conducting  $I_{Kr}$ , as many chemicals and biological products have a high affinity for these channels.<sup>46</sup> Blockade of this channel decreases  $I_{Kr}$ , which prolongs the repolarization phase and thus APD. During this period of prolongation,  $I_{Na}$  and  $I_{Ca,L}$  can flow due to re-activated channels, giving rise to spontaneous early after depolarizations (EADs), often a characteristic of ectopic activity which is considered being pro-arrhythmic. Due to this, electrical activation can occur at locations other than the sinus node, indicating by an electrical storm on the ECG. Fortunately, under physiological conditions other potassium currents can compensate for the lack of, for example,  $I_{Kr}$ , which is considered as a ‘repolarization reserve’ of the myocardium.

Although prolonged existence of sodium or calcium currents during the repolarization can cause EADs, SR calcium leakage via RyR or calcium influx via Cx43 hemichannels during the resting phase can cause delayed afterdepolarizations (DADs).<sup>47,48</sup> While DADs can

trigger ectopic activity similar to EADs, the premature activation of cardiac tissue can also cause electrical conduction block, which is, similar to a physical conduction block by increased collagen deposition, an instigator of reentry.

## 4 | ARRHYTHMOGENICITY IN CHRONIC KIDNEY DISEASE

Alternative disturbances introduced by CKD that affect cardiac electrophysiology are also important pro-arrhythmic factors. In particular, imbalance in serum potassium concentrations and autonomic nervous system dysfunction indirectly can also partially be contributed to the accumulation of UTs. Before describing clinical and experimental research on cardiac electrophysiology induced by UTs, these mechanisms will be described to fully cover arrhythmogenicity in CKD.

### 4.1 | Hypokalemia and hyperkalemia

High intracellular and low extracellular potassium are the main determinants of the negative resting membrane potential, which prevents spontaneous excitability of cardiomyocytes. Simultaneously, this balance establishes an outward driving force for potassium currents. Fluctuation of serum potassium concentrations



to abnormal low and high extremes is a problematic clinical manifestation, especially in CKD patients.<sup>49</sup> The kidney plays a pivotal role in the regulation of serum potassium levels, with a diminished kidney filtration being a predictor of hyperkalemia ( $>5.0$  mEq/L potassium).<sup>50</sup> Additionally, nutrition, medication, and dialysis also significantly influence serum potassium<sup>51</sup> and can sway it to both hyperkalemia and hypokalemia ( $<4.0$  mEq/L potassium). Interestingly, while hyperkalemia is a widely acknowledged issue in CKD, the prevalence of both hyper- and hypokalemia is similar and estimated around 12–20% in the CKD population.<sup>49,50</sup> Consequently, alterations in potassium metabolism can result in detrimental cardiac rhythm abnormalities. As in ESRD patients, blood volume and electrolyte levels alternate often after dialysis, this could partly explain the high incidence of arrhythmias in that population.<sup>5,18</sup>

During hypokalemia, the resting membrane potential is hyperpolarized, and a larger outward driving force for potassium is present. However, repolarization currents such as  $I_{K1}$  and  $I_{Kr}$  have alternate methods of regulation which result in a decreased outward current.  $I_{K1}$  is naturally blocked by voltage-dependent polyamines and magnesium, which in turn can be removed by extracellular potassium.<sup>52</sup> Low extracellular potassium would therefore stabilize  $I_{K1}$  block. The inactivation rate of  $I_{Kr}$  as well as its ion channel expression are influenced by potassium, with hypokalemia resulting in enhanced inactivation and decreased expression.<sup>53,54</sup> These decreased currents lower the repolarization reserve and prolong the APD, thereby increasing susceptibility to EADs and triggered activity.<sup>55</sup> Alternatively, the hyperpolarization of the membrane potential and lowered extracellular potassium inhibit NKA activity. This leads to increased cytosolic sodium, which diminishes NCX activity, eventually leading to increased cytosolic calcium.<sup>56</sup> Ultimately, this calcium overload may induce DADs and associated rhythm abnormalities.

Opposite to that, during hyperkalemia the resting membrane potential depolarizes, initially leading to increased excitability due to the potential being closer to the activation potential of  $I_{Na}$ . However, the inactivation of sodium channels also increases at depolarizing potentials, leading to less channels contributing during every depolarization. Supplementary and opposed to hypokalemia, the repolarization reserve is increased, with a shorter APD as the consequence. Irregular activation of sodium channels and a chronic increase of the repolarization reserve can prolong the period of inexcitability of cardiomyocytes. In combination, this culminates into altered excitation, electrical recovery, and decreased conduction between cardiomyocytes,<sup>55,57</sup> which in turn can lead to conduction block and reentrant arrhythmias.

## 4.2 | Autonomic nervous system dysfunction

The communication between the autonomic nervous system (ANS) and the kidneys is crucial for an appropriate kidney function, for example, regulating blood osmolarity and maintaining the sodium balance.<sup>58</sup> The direct sympathetic innervation of the heart is mainly mediated by norepinephrine in concert with circulating adrenaline being produced by the adrenal gland, both targeting adrenergic receptors. Elevated sympathetic stimulation thereby leads to an increased heart rate and contractility. This is achieved via the effects on the sinoatrial node and the individual cardiomyocytes,<sup>59,60</sup> where cyclic adenosine monophosphate (cAMP) enhances ion channel activity and other positive inotropic effects after adrenergic stimulation. This is counteracted by the parasympathetic nervous system, through acetylcholine binding to muscarinic receptors. A major player in autonomic modulation is acetylcholine signaling, being instigated by nitric oxide (NO).<sup>61</sup> cGMP levels are mediated by NO, increasing cAMP breakdown via phosphodiesterases, resulting in negative inotropic effects.<sup>62</sup>

During CKD, NO bioavailability is reduced, due to both increased oxidative stress and increasing asymmetric dimethylarginine (ADMA) levels,<sup>59,63,64</sup> which results in a sympathetic overdrive.<sup>64,65</sup> This is especially observed in ESRD patients.<sup>66</sup> While oxidative stress can be promoted by elevated UT levels, UTs are also presumed to directly increase sympathetic activity by inducing inflammation and NO deficiency in the central nervous system.<sup>59</sup> While further electrophysiological mechanisms are scarcely available in the setting of CKD, examples of increased NCX activity providing spontaneous calcium triggers are also observed.<sup>67</sup> Augmented sympathetic activity can cause, or aggravate, existing rhythm disturbances leading to arrhythmias such as AF,<sup>60,68,69</sup> again partially explaining high SCD rates in the ESRD and dialysis populations.<sup>36–38</sup>

## 5 | CURRENT CLINICALLY ORIENTATED UREMIC TOXIN RESEARCH

The correlations between increased levels of PBUTs and diminished kidney function as well as cardiac dysfunction have been well established.<sup>12,20,32</sup> Therefore, research on those UTs has mainly been focused on improving clinical outcome by adapting dialysis, but also through additionally decreasing UT concentrations in ESRD patients, through instrumentation of adsorbent and displacing chemicals. Investigations regarding the improvement of the applied dialysis method range from adjusting speed and frequency of dialysis sessions, to adjustments of the

dialysate or physical conditions within the dialyzer.<sup>70</sup> Additionally, alternative filtration techniques such as hemodiafiltration are studied to improve removal of PBUTs, for example, in the recently initiated CONVINCE study.<sup>71</sup>

## 5.1 | Clinical improvements

The addition of liposomes to the regimen of dialysis has experimentally been shown to increase the removal of PBUTs.<sup>72,73</sup> These liposomes remain in the dialysate and act as adsorbent, taking up PBUTs. While still in the experimental phase, the natural components of the technique offer a potentially safe and efficient addition to dialysis. The adsorptive effect of sevelamer, already prescribed for prevention of hyperphosphatemia in CKD patients, was also investigated in a trial with advanced staged CKD patients.<sup>74</sup> However, a 12-week treatment with sevelamer did not show a decline in concentrations of IS, pCS, and IAA. Another well-investigated chemical adsorbent is AST-120 (Kureha Company, Japan).<sup>75–78</sup> AST-120 is an oral carbon adsorbent that binds UTs and retains them in the intestines, to decrease their concentrations in the circulation by limiting their uptake. It has been shown to effectively decrease concentrations of, for example, IS and pCS in patients,<sup>75</sup> subsequently followed by decreased concentrations of IL-1 $\beta$  and NF $\kappa$ B.<sup>79</sup> While it's not for optimal clearance of all UTs, AST-120 remains one of the most effective methods of reducing UT concentrations,<sup>80,81</sup> but the effect on CKD progression remains uncertain thus far.<sup>82,83</sup>

Alternative to adsorbents are displacing agents like ibuprofen, which shares the binding site for albumin with PBUTs.<sup>84</sup> By displacing the UTs on albumin, their free fraction increases, allowing a more easily filtration during regular hemodialysis. Unfortunately, the high concentration of ibuprofen that is needed to increase the free fraction of, for example, IS and pCS just 3-fold, can result in additional complications in CKD patients.<sup>85</sup> On top of that, the binding site is not shared with all PBUTs, meaning that several displacing agents are needed for the entire set of PBUTs. Other more recently investigated displacing agents are salvianolic acids.<sup>86</sup> While their mechanistic concept is similar to that of ibuprofen, the most effective salvianolic acids share a different binding site for albumin, which could also be beneficial. However, these chemicals also need to be cleared by the kidneys after their administration, which eventually could become detrimental for CKD patients.

As many UTs are dietary metabolites produced in the intestines, decreased protein intake and plant-based diets result in lower UT concentrations, potentially caused by modification of the gut microbiota.<sup>87–89</sup> Therefore, supplementation of the gut microbiota using probiotics

to decrease UT production has also been investigated. Unfortunately, the supplement did not lower UT concentrations, but adversely increased serum potassium and urea levels.<sup>90</sup> In conclusion, some additional clinical modes of intervention, and multiple adsorbents and displacers are capable to improve PBUT filtration from the circulation. However, no methods have been established yet that are both safe and efficient.<sup>85</sup> A more complete understanding of specific PBUTs and their contribution to disease pathology could aid in targeting their effects in patients.

## 6 | CURRENT ELECTROPHYSIOLOGICAL RESEARCH

Arrhythmogenicity as the consequence of pathological cardiac electrical remodeling is rarely investigated in experimental studies of CKD, especially regarding the affected electrophysiological mechanisms which are at the basis of the arrhythmias seen in patients.<sup>23,91</sup> In vivo experiments mainly consist of ECG data, sometimes with a follow-up on cellular electrophysiology, while in vitro studies that are considered as completely cell culture-based experiments are mostly lacking. Despite of that they would allow for a more detailed investigation into pathophysiology of single UTs. Mouse and rat models are often used for their reasonably low housing costs and increased availability compared to larger animals. While cardiomyocytes from these animals can technically be used very well for electrophysiological experiments, their species-specific electrophysiology significantly differs from human cardiomyocytes, whereas cellular electrophysiology in rabbits, dogs, and pigs is much more similar to humans.<sup>92</sup> An overview of studies that specifically included relevant parameters for arrhythmogenicity in CKD is shown in [Table 1](#) (in vivo, see [Section 6.1](#)) and [Table 2](#) (in vitro, see [Section 6.2](#)).

### 6.1 | In vivo CKD studies on cardiac electrophysiology

The most common approach to establish animal models of CKD is nephrectomy, either subtotal (SNx) or unilateral (UNx), removing 5/6 or 1/2 of the kidneys, respectively.<sup>93–100</sup> This abrupt reduction in nephron numbers causes kidney insufficiency, often followed by an inflammatory response and fibrosis formation, potentially systemically. Another approach is genetically inducing CKD, either by causing a defect in the *Samcystin* gene, causing polycystic kidney disease (Cy/+),<sup>101,102</sup> or a defect in type 4 collagen alpha-3, resulting in increased FGF23 levels and renal fibrosis (Col4a3<sup>-/-</sup>).<sup>103</sup> Both initiate the

TABLE 1 Studies on the effect of CKD and uremic toxins on cardiac electrophysiology in vivo

Animal	CKD	Toxin	Outcome	References
Mouse	DOCA	–	Increased occurrence of arrhythmia, decreased conduction velocity, decreased Cx43 expression	[93]
Mouse	SNx	–	Increased occurrence of arrhythmia, decreased Cx43 expression	[93]
Mouse	SNx	–	No contractile dysfunction Hampered calcium handling	[94]
Rat	SNx	–	Prolonged QTc, increased ventricular arrhythmogenesis Hampered calcium handling	[95]
Rat	UNx	–	Prolonged QTc Increased action potential duration	[96]
Rat	SNx	–	Increased occurrence of atrial fibrillation Cx43 redistribution Fibrosis formation	[97]
Rabbit	SNx	–	Increased occurrence of atrial arrhythmia Increased LA action potential duration and fibrosis formation	[98]
Rabbit	SNx	–	Increased ventricular arrhythmogenesis, fibrosis formation	[99]
Dog	SNx	–	Prolonged QTc, increased hypertrophy	[100]
Rat	Cy/+	–	Increased occurrence of arrhythmia and sudden cardiac death	[101]
Rat	Cy/+	–	Increased action potential duration, increased occurrence of ventricular fibrillation, hampered calcium handling	[102]
Mouse	Col4a3 <sup>-/-</sup>		Contractile dysfunction, hampered calcium handling	[103]
Mouse	–	FGF23	Hampered calcium handling	[94]

substitution of functional kidney tissue, either with or by increased collagen deposition. These CKD models show pro-arrhythmic parameters such as prolonged QTc, single cell APD prolongation, slowed conduction velocity, and increased tachyarrhythmias. Interestingly, mechanistic investigations always indicate a hampered calcium handling, fibrosis formation, and a decreased Cx43 expression as the main potentiators of the pro-arrhythmic parameters.<sup>93–95,97–99,102,103</sup> Perfusion of a single toxin is an alternative method to specifically investigate its effects, which in case of the non-toxin FGF23 revealed to lead to increased arrhythmogenicity, via disturbed calcium handling.<sup>94,104</sup>

Unfortunately, the main drawback of these studies is that there are no data available on specific culprits for the electrophysiological changes and the mechanisms behind the effects have rarely been investigated. Ultimately, establishing a CKD model with consistent electrophysiological remodeling would be highly beneficial in the search for pharmacological interventions to treat or prevent the occurrence of arrhythmias.

## 6.2 | In vitro CKD studies on cardiac electrophysiology

To study the maladaptive effects of individual UTs preferably should also be performed in in vitro experiments, where

no systematic disease can be instigated that would influence the results. This has been performed, to a limited extent, in cardiomyocytes isolated from animals and in immortal cell lines (e.g. H9c2).<sup>48,105–110</sup> IS has been shown to cause Cx43 disruption, leading to a compromised intercellular gap junction communication.<sup>48,105</sup> IS and pCS both decrease repolarizing potassium currents in a dose-dependent manner,<sup>107,108</sup> which was subsequently proposed to prolong the APD in computer simulations.<sup>108</sup> Chronic exposure to FGF23 can lead to calcium leakage from the SR, giving rise to uncontrolled calcium waves and contractions.<sup>109,110</sup>

In general, these alterations in conduction properties, AP formation and calcium handling, represent the pro-arrhythmogenicity corresponding to results from in vivo studies. Application of single toxins on cell cultures should be used to augment knowledge on molecular changes caused by each toxin, in parallel to striving for development of effective interventions.

## 7 | FILLING THE GAP IN KNOWLEDGE: FUTURE EXPERIMENTAL STUDIES

Currently, CKD patients are highly prone to cardiac arrhythmias, without existing preventive therapies being available. In the last two decades, clinical CVD treatment



TABLE 2 Studies on the effect of CKD and uremic toxins on cardiac electrophysiology in vitro

Cell type	CKD	Toxin	Outcome	References
Rat neonatal cardiomyocytes	–	Indoxyl sulfate	Cx43 redistribution, disruption of gap junction communication	[105]
Rat neonatal cardiomyocytes	–	P-cresol	Cx43 disassembly, disruption of gap junction communication	[106]
Rat ventricular cardiomyocytes (H9c2)	–	P-cresyl sulfate	Dose-dependent decrease of $I_{Kr}$	[107]
Rat ventricular cardiomyocytes (H9c2)	–	Indoxyl sulfate	Dose-dependent decrease of $I_{Kr}$	[108]
Rat adult ventricular cardiomyocytes	–	FGF23	Increased occurrence of spontaneous calcium waves, SR calcium leak	[109]
Rat adult ventricular cardiomyocytes	–	FGF23	Increased occurrence of spontaneous calcium waves, contractile dysfunction, decreased L-type calcium current	[110]
Rabbit atrial and pulmonary vein cardiomyocytes	–	Indoxyl sulfate	PV: Increased afterdepolarizations, SR calcium leakage Atrial: increased occurrence of fibrillations	[48]

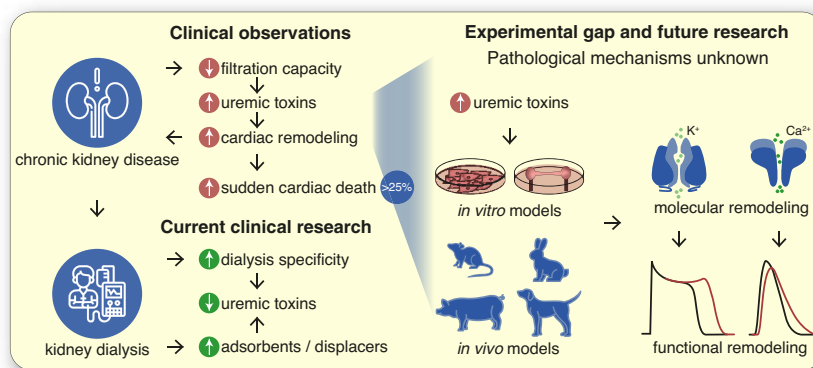
in CKD patients has improved importantly, with the exception of SCD.<sup>111</sup> Clinically orientated research and experience show only treatments of symptoms, like application of antiarrhythmic drugs, atrial ablation strategies, and implantable cardioverter-defibrillator (ICD) therapy.<sup>91</sup> However, with limited understanding of the pathological mechanisms behind SCD in CKD patients, the applied clinical approaches to control deterioration of the disease remain rather non-specific and not always successful, as has been seen in the discontinued ICD2 trial.<sup>112</sup> In this trial, it was shown that prophylactic ICD implantation in dialysis patients did not decrease SCD occurrence, but resulted in an additional increased risk of adverse events related to the procedure of implantation. A major advantage for clinical progress would be a detailed and in-depth knowledge of UTs and their specific effects on the heart. Low-protein diets decrease the production of UTs, but they remain high in serum concentrations, especially in dialysis patients. Pro-fibrotic characteristics of UTs have already been investigated more extensively, but electrophysiological knowledge on the cause of ventricular arrhythmia triggering SCD remains elusive.<sup>12,15,23</sup>

To design future electrophysiological experiments, clinically relevant UT concentrations should also be applied in vitro. Based on the overview of PBUTs and their respective total plasma concentrations as shown in Figure 2, an experimental concentration range (shown in blue) can be established, covering both clinical conditions. By doing this, not only effects of UTs can be investigated, but more refined clinical concentration boundaries can also be established. Importantly, in experimental designs of PBUTs it is of relevance to include 35–50 gr/L albumin in the experimentally applied protocols, as only the free fraction is effective,<sup>113</sup> which is established after including albumin with the concentrations as depicted in Figure 2.

To specifically investigate electrophysiologically relevant parameters, such as APD and calcium handling and their underlying mechanisms, experimental models should be carefully selected. While conduction disorders and rhythm abnormalities can be explored in mice, the proposed approach for mechanistic background studies is preferably performed in cardiomyocytes from rabbit, dog, or pig hearts, especially regarding repolarization of the action potential.<sup>92</sup> Moreover, the differences in natural sympathetic drive in different animals should be acknowledged. Beneficial in these in vivo models is the analysis of systemic effects after CKD initiation, on top of the subsequent cellular analysis. However, this method only allows to study the overall effects of CKD on cardiac electrophysiology, thereby still not elucidating the effects of individual UTs. Due to costs, availability, and ethical constraints for animal studies, it is of significant value to design and improve relevant in vitro models.

Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) are often used as alternative electrophysiological relevant cell system. iPSC-CMs are spontaneously active, contract, and parameters such as action potentials, calcium transients, and individual ion currents can be measured, with additional protein and gene analysis that can further explore the effects caused by exposure to individual or cocktails of clinically relevant concentrations of UTs. Drawback of these models is that iPSC-CMs still have a rather immature electrical and morphological phenotype, which requires a skeptical perspective on their translational capabilities.<sup>114</sup> Additionally, preparation, culture, and differentiation of these cells remain generally inconsistent, lowering output both qualitatively and quantitatively.

Robust steps in the field of tissue engineering have led to improved maturation and applicability of iPSC-CMs,



**Central Illustration.** Fundamental gap between clinical and experimental research of the cardiorenal syndrome, focused on cardiac electrophysiology and arrhythmogenesis. Development of a worsened cardiac function due to chronic kidney disease, leading to sudden cardiac death in approximately 25% of the latest stage chronic kidney disease patients. Clinical research mainly focusses on direct treatment options to reduce uremic toxins as important regulator in the cardiorenal syndrome. Experimental studies investigating the mechanisms underlying electrophysiological remodeling are lacking, complicating efficient research targeting treatment improvements.

producing engineered heart tissues (EHTs) and ventricular heart chambers.<sup>115-117</sup> EHTs can consist of combinations of multiple iPSC-derived cell types, such as cardiomyocytes, fibroblasts, and endothelial cells, and are much more reproducible compared to regular and commonly used (2-dimensional, only cardiomyocytes) cultures of iPSC-CMs. Contraction of such constructs is unidirectional, improving cardiomyocyte orientation.<sup>118</sup>

## 8 | CONCLUSION

This review describes the fundamental knowledge gap of the effects of uremic toxins on cardiac electrophysiology and arrhythmogenesis (see Central Illustration). The accumulation of UTs during advancing stages of CKD, especially PBUTs that are not filtered during dialysis, proved to be maladaptive with regard to cardiac structural remodeling. Apprehension of the involvement of UTs in remodeling of cardiac electrophysiology remains scarce, despite high prevalence of arrhythmias and SCD in CKD patients. Clinical UT research focusses mainly on adapting and improving dialysis. In contrast, pro-arrhythmic molecular mechanisms evoked by individual or combinations of UTs require unmet detailed investigations, preferably in state-of-the-art engineered cardiac tissues and relevant animal models.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest nor relevant relations with industry.

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